RNA Isolation from Pico-scale samples with the PicoPure ™

- **Protocol for Use with CapSure™ Macro LCM Caps**
- **Protocol for Use with <10mg Frozen Tissue**

Columns can handle: 1 cell (10-25pg) – 10mg tissue
Yields of total RNA: 0.001ng –100µg

Materials

PicoPure™ RNA Isolation Kit, Arcturus # KIT0202 / KIT0204, exp. date: 6 months

Reagents and Supplies in kit:

Item	Vial Name
Conditioning Buffer	**CB**
Extraction Buffer	**XB**
70% Ethanol	**EtOH**
Wash Buffer 1	**W1**
Wash Buffer 2	**W2**
Elution Buffer	**EB**
RNA purification columns with collection tubes	
Microcentrifuge tubes	

RNase-Free DNase Set, Qiagen #79254

Prepare DNase I stock solution before using for the first time:
Dissolve the solid DNase I (1500 Kunitz units) in 550µl RNase-free water.
Mix gently by inverting tube (do not vortex)
Divide the stock solution into single-use aliquots, and store -20^{o}C for up to 9 months.
Thawed aliquots can be stored at 2-8^{o}C for up to 6 weeks. Do not refreeze aliquots after thawing.

Microcentrifuge
2 – 20µl pipettor
20 – 200µl pipettor
100 – 1000µl pipettor
Incubation oven

Recommendations for Storing RNA

Cell extracts resulting from completion of Part I may be stored at –80^{o}C.
Use isolated RNA immediately for amplification, or store at –80^{o}C for up to 6 months.

Methods

1. **RNA Extraction**
    a. **For LCM sample**
        i. Mix XB. If precipitate is present, warm and mix.
        ii. Pipette 50µl into a 0.5 ml tube.
        iii. Insert and invert CapSure Macro LCM Cap-µtube assembly.
        iv. Incubate assembly at 42^{o}C for 30 min.
        v. Invert and centrifuge assembly at 800 x g for 2 min.

vi. Remove the CapSure Macro LCM Cap and save the microcentrifuge tube with the cell extract in it.
vii. Proceed with RNA isolation protocol or freeze cell extract at –80^{o}C.

b. **For Frozen Pico-scale samples**
   i. Add 50µl XB to tube containing frozen tissue
   ii. Briefly vortex
   iii. Incubate 42^{o}C, 30min, 500rpm
   iv. Centrifuge 3,000 x g, 2min
   v. Pipette supernatant to new tube (avoid pick-up of pelleted material)

2. RNA Isolation

a. Pre-condition the RNA Purification Column:
   i. Pipette **250µl CB** onto the purification column filter membrane.
   ii. Incubate for **5 min at RT**.
   iii. Centrifuge the column at 16,000 x g for 1 min.
b. Pipette equal volume **(50 µl) of 70% EtOH** into the cell extract from Part I. Mix well by pipetting up and down. DO NOT CENTRIFUGE.
c. Pipette the cell extract and EtOH mixture into the pre-conditioned purification column (~100µl).
d. To bind RNA to the column, centrifuge at **100 x g for 2 min**, immediately followed by centrifugation at **16,000 x g for 30 sec** to remove flowthrough.
e. Pipette **100µl W1** into the column and centrifuge at **8,000 x g for 1 min**.
f. DNase treatment
   i. Pipette **40µl DNase solution mix** directly into the purification column membrane.
      - For LCM and small samples: 5µl DNase I Stock solution + 35µl Buffer RDD. Mix by gently inverting.
      - For larger (pico-scale) samples: 10µl DNase I Stock solution + 30µl Buffer RDD. Mix by gently inverting.
   ii. **Incubate RT, 15 min**.
   iii. Pipette **40µl W1** into the column and centrifuge **at 8,000 x g for 15 sec.**
g. Pipette **100µl W2** into the column and centrifuge at **8,000 x g for 1 min**.
h. Pipette **100µl W2** into the column and centrifuge at **16,000 x g for 2 min**.
i. Discard flowthrough waste and recentrifuge the column at **16,000 x g for 1 min** to remove all traces of wash buffer prior to elution step.
j. Transfer the purification column to a new 0.5ml microcentrifuge tube (provided in kit).
k. Pipette **12µl EB** directly onto the membrane of the purification column (gently touch the tip of the pipette to the surface of the membrane while dispensing the elution buffer to ensure maximum absorption of EB into the membrane).
l. Incubate the purification column at **RT for 1 min**.
m. Centrifuge the column at **1,000 x g for 1 min** to distribute EB in the column, then at **16,000 x g for 1 min** to elute RNA.
   ••to avoid potential breakage of the microcentrifuge tube cap during centrifugation, insert the pur. column/0.5ml tube assembly into a lidless 1.7/2.0ml tube. Insert this assembly into adjacent rotor holes as illustrated. Rest the tube cap against the tube immediately clockwise to it. Place an

empty lidless 1.7/2.0ml tube into the rotor hole adjacent in the clockwise direction to the last assembly.

3. **RNA Concentration**
   Quantify using NanoDrop. Use a known RNA sample to verify NanoDrop is measuring accurately.

4. **RNA Quality**
   Submit sample (500-1000pg/µl) to NMG for BioAnalyzer using PicoChip.